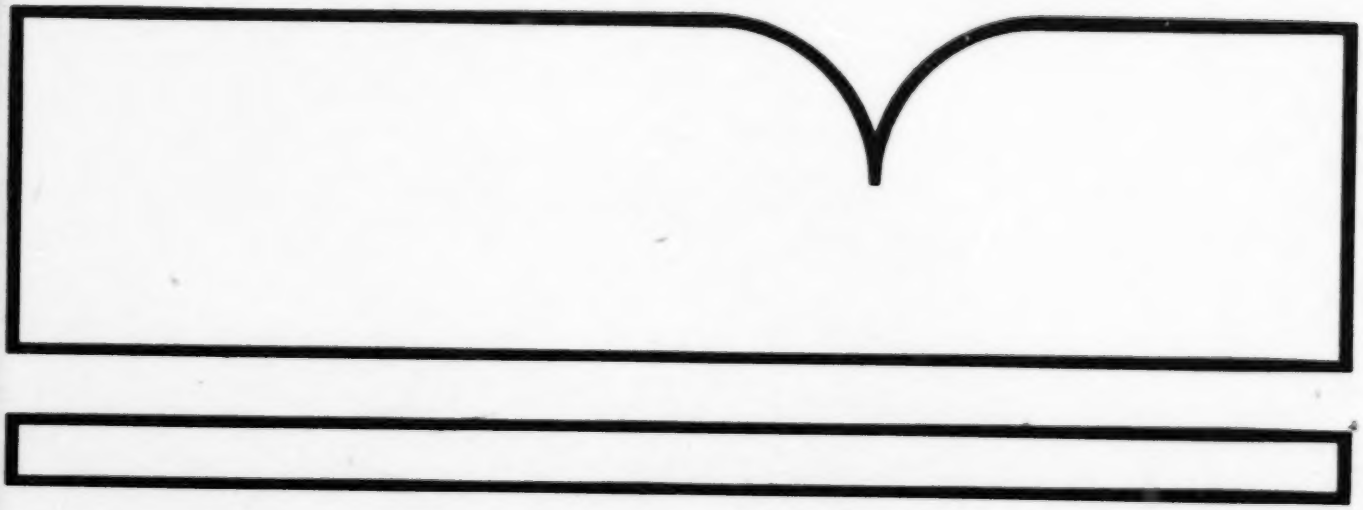
PB81-212128

Induction of Skin Papillomas in the Sencar Mouse as a Tier 2 Carcinogenesis Bioassay

(U.S.) Health Effects Research Lab.
Cincinnati, OH

1981



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TECHNICAL REPORT DATA
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1. REPORT NO.	2.	3. RECIP
EPA-600/J-81-189	Journal Article	PB81-212128

4. TITLE AND SUBTITLE	5. REPORT DATE
Induction of Skin Papillomas in the Sencar Mouse as a Tier 2 Carcinogenesis Bioassay	May 1981
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) R.J.Bull, M.Robinson, J.Glass, D.Cmehil, J.Orthoefer	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. EPA Health Effects Research Laboratory 26 W. St. Clair St. Cincinnati, Ohio 45268	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Same	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES

Published as an Abstract in: Toxicologist 1:129-130 (1981)

16. ABSTRACT

The Toxic Substances Control Act mandates the testing of industrial chemicals for which insufficient evidence of safety exists. One of the more critical areas in chemical carcinogenesis testing is a dependable approach to confirmatory tests (tier 2) of identified positives at a screening level (tier 1). The present work was undertaken as part of a systematic evaluation of a series of short-term in vitro and in vivo tests which possess properties thought desirable for tier 2 testing. Experiments were designed to determine whether the mouse skin responds to initiating doses of carcinogens selected from the major carcinogen classes administered by four routes, oral, IP, Sub Q and topical. Experiments to date include testing of diethylnitrosamine, urethane, benzene, benzo(a)pyrene, DMBA, aflatoxin B_1, methylmethane sulfonate, azobenzene, FANFT and lead acetate. Two weeks following application of the initiating doses of each carcinogen a 20 week promotion schedule involving the application of 1µg of 12-0-tetradecanoyl phorbol-13-acetate (TPA) was initiated. On the basis of preliminary data it is apparent that papilloma development in response to chemical carcinogens varies with route of exposure. For example, it is apparent that the response to urethane administered orally exceeds that observed by the topical route by 3-fold. Conversely, the response of the mouse skin to benzo(a)pyrene is more than 10-fold by the topical vs. systemic routes of exposure. Route specific responses to DMBA, benzene, diethylnitrosamine and aflatoxin B_1 are also apparent.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to Public	Unclassified	
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
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EPA-600/J-81-189
Journal Article

The Toxicologist

An official publication of the Society of Toxicology.

VOL. 1 NO. 1 MARCH 1981

ClCH₂CH-CHCl-O-CH₂CH₂-Cl

International Congress '82

found in the ethmoturbinates. The tumor invaded nasal bone (48%) and brain (2.6%). Tumor metastasis to the lung (5.4%) and cervical lymph nodes (1.2%) were observed. The epidermoid carcinoma often revealed glandular tumor cells. The tumors originated from both nasal epithelium and glands showing squamous metaplasia with hyperkeratosis. Electron microscopy revealed mixed characteristics of both the glandular and epidermoid traits in the tumor cell indicating the capacity of tumor cells to shift from a glandular to a squamous differentiation.

467 RESULTS OF A CHRONIC TOXICITY AND ONCOGENICITY STUDY IN RATS MAINTAINED ON WATER CONTAINING ACRYLONITRILE FOR 24 MONTHS. J.F. Quast, C.G. Humiston, C.E. Wade, R.M. Carreon, E.A. Hermann, C.N. Park, and B.A. Schwetz, Dow Chemical H&ES, USA, Midland, MI 48640.

A 24-month industry supported study was conducted in Sprague-Dawley, Spartan substrain rats to assess the chronic toxicity and oncogenicity of acrylonitrile (AN) when administered in their drinking water. Nominal concentrations of AN were 0 ppm (C), 35 ppm (LD), 100 ppm (MD), or 300 ppm (HD). These levels of AN in their drinking water provided an equivalent mean dosage of 0, 4, 9, or 22 mg/kg/day.

There were dose-related decreases in water consumption, food consumption, and weight gain in both sexes of rats at all dose levels of AN in the drinking water. The rats appeared unthrifty, exhibited early mortality, and had an early onset of palpable tumors. Due to early mortality in the treated rats many of the pathologic changes seen in aged rats were observed less frequently than in controls. Endocardial fibrosis was observed with increased frequency in the HD male group of rats.

An increased tumor incidence was observed in treated rats and affected the central nervous system, ear canal gland (Zymbal gland), stomach (nonglandular portion), tongue, small intestine, and mammary gland. In addition, a decreased incidence of tumors was also observed in the treated rats and involved the pituitary gland, thyroid gland, adrenal glands, pancreas, and uterus. Of the various organs in male and female rats which exhibited an oncogenic response at all treatment levels of AN, the nervous system appeared to be most affected. Evidence of toxicity and oncogenicity was observed in rats at all dose levels of AN used in this study.

468 CHRONIC TOXICITY AND RISK ASSESSMENT IN MICE EXPOSED TO BENZIDINE DIHYDROCHLORIDE. N.A. Littlefield, C.J. Nelson, & G. Schieferstein, National Center for Toxicological Research, Jefferson, Arkansas

Benzidine, recognized as a bladder carcinogen in man and as a liver carcinogen in experimental animals, is the chemical basis of as many as 200 commercial dyes. It has recently been demonstrated that physiological processes can metabolize these dyes to release benzidine, thereby creating a potential exposure hazard. To assess this hazard, both sexes of F_1 hybrid (homogeneous) and F_2 (heterogeneous) mice from a BALB/c male and C57BL/6 female cross were exposed for their respective life spans to benzidine dihydrochloride in their drinking water at concentrations of 0, 30, 40, 60, 80, 120, and 160 ppm for males and 20, 30, 40, 60, 80, and 120 ppm for females. Animals were removed from the study only when they were dead or moribund. This study was terminated after 33 months of exposure. Using the endpoint of hepatocellular adenomas and carcinomas, the Armitage-Doll multistage model was used to describe the tumor rates in the experimental dose range and obtain the upper confidence level on tumor rates. Linear interpolation was used between 0 dose and the upper confidence level of the lowest experimental dosage for predicting potential low dose tumor rates. Dose response effects on body weight, survival, and liver neoplasms were noted in both strains. For each of the endpoints, the females were more susceptible than males and the F_1 (homogeneous) strain was more susceptible than the F_2 (hetergeneous) strain. The calculated virtually "safe" dose predicted to produce less than one per million F_1 female mice with a liver tumor is 0.04 ppb. One ppb of benzidine dihydrochloride in the drinking water of these mice would theoretically produce liver tumors in less than 2.49 mice per 100,000 population.

469 CARCINOGEN BIOASSAY OF DI(2-ETHYLHEXYL)PHTHALATE. J.F. Douglas and W.V. Hartwell, National Toxicology Program/NCI, NIH, Bethesda, MD 20205.

A carcinogen bioassay of di(2-ethylhexyl)phthalate, the most commonly used plasticizer for polymers, was conducted in rats and mice. The compound was administered orally in feed for two years at 6000 and 12000 ppm to both sexes of F344 rats and at dose levels of 3000 and 6000 ppm to both sexes of B6C3F1 mice. Group sizes were 50 including matched controls.

Di(2-ethylhexyl)phthalate induced increased incidence of hepatocellular carcinomas or adenomas in both male and female mice, and it also significantly increased hepatocellular carcinomas or neoplastic nodules in either sex of rats. The chemical also produced atrophy and tubular degeneration of the testes and hypertrophy of anterior pituitary cells in male rats.

It was concluded that di(2-ethylhexyl)phthalate was carcinogenic to the F344 rat and the B6C3F1 mouse under the experimental conditions.

470 INDUCTION OF SKIN PAPILLOMAS IN THE SENCAR MOUSE AS A TIER 2 CARCINOGENESIS BIOASSAY. R.J. Bull, M. Robinson, J. Glass, D. Cmehil and J. Orthoefer, Health Effects Research Laboratory, USEPA, Cincinnati, Ohio 45268

The Toxic Substances Control Act mandates the testing of industrial chemicals for which insufficient evidence of safety exists. One of the more critical areas in chemical carcinogenesis testing is a dependable approach to confirmatory tests (tier 2) of identified positives at a screening level (tier 1). The present work was undertaken as part of a systematic evaluation of a series of short-term in vitro and in vivo tests which possess properties thought desirable for tier 2 testing. Experiments were designed to determine whether the mouse skin responds to initiating doses of carcinogens selected from the major carcinogen classes administered by four routes, oral, IP, Sub Q and topical. Experiments

to date include testing of diethylnitrosamine, urethane, benzene, benzo(a)pyrene, DMBA, aflatoxin B_1, methylmethane sulfonate, azobenzene, FANFT and lead acetate. Two weeks following application of the initiating doses of each carcinogen a 20 week promotion schedule involving the application of 1 ug of 12-O-tetradecanoyl phorbol-13-acetate (TPA) was initiated. On the basis of preliminary data it is apparent that papilloma development in response to chemical carcinogens varies with route of exposure. For example, it is apparent that the response to urethane administered orally exceeds that observed by the topical route by 3-fold. Conversely, the response of the mouse skin to benzo(a)pyrene is more than 10-fold by the topical vs. systemic routes of exposure. Route specific responses to DMBA, benzene, diethylnitrosamine and aflatoxin B_1 are also apparent. These results suggest that consideration be given to employing more than one route of exposure in testing of chemicals using the mouse skin initiation/promotion system.

471 CARCINOGEN BIOASSAYS OF SEVERAL PHTHALIC ACID DERIVATIVES AND RELATED COMPOUNDS. W.V. Hartwell and J.F. Douglas, National Toxicology Program/NCI, NIH, Bethesda, Maryland 20205.

Carcinogenesis bioassays have been conducted with select phthalic acid derivatives and di(2-ethylhexyl)adipate. Test animals were F344 rats and B6C3F1 mice. Only those esters containing the 2-ethylhexanol moiety were considered carcinogenic. Hepatocellular carcinomas occurred in a dose related fashion in high- and low-dose male and female rats and mice which received diets containing the di(2-ethylhexyl)phthalate. Di(2-ethylhexyl)adipate was not carcinogenic in rats; however, increased incidences of liver tumors were detected in both male and female mice. In separate studies, increased tumor incidences were not detected in male or female Wistar or Sherman rats fed diets containing di(2-ethylhexyl)phthalate for 104 weeks.

472 CARCINOGENICITY OF INHALED 1, 2-DIBROMOETHANE IN RATS TREATED WITH DISULFIRAM. L. C. K. Wong, J. M. Winston, and C. B. Hong, Midwest Research Institute, Kansas City, MO.

A chronic inhalation study of ethylene dibromide (EDB) was conducted in Sprague Dawley rats. The study consisted of four groups of rats receiving control air and control diet, 20 ppm of EDB and control diet, control air and 0.05% of disulfiram (DS) in the diet, and 20 ppm of EDB and 0.05% of DS in the diet, over a period of 18 months. Rats receiving 20 ppm of EDB showed high mortality and an increase in tumor incidences in mammary glands, spleen, adrenals, liver, kidneys, and subcutaneous tissues. A combination of 20 ppm of EDB and 0.05% DS in the diet caused earlier deaths and high mortality rate, decreases in body weight gain, food consumption, hemoglobin, hematocrit and RBC counts in rats. This combination of EDB and DS treatment also caused early development and high incidences of tumors in liver, spleen, kidneys, adrenals, thyroids, lungs, mesentary and mammary tumors. In addition, 90% of male rats that received a combination of EDB and DS treatment had testicular atrophy. Addition of DS treatment caused early deaths and high mortality rate, and promoted not only the tumor incidences in various organs and tissues in both male and female rats, but also the testicular atrophy in rats.

473 TOXICOLOGICAL EVALUATION OF CARMINOMYCIN - AN ANTINEOPLASTIC ANTHRACYCLINE ANTIBIOTIC. R. A. Buroker, D. F. Johnson and H. Madissoo, Department of Toxicology, Bristol Laboratories, Syracuse, N.Y.

Carminomycin, an anthracycline antibiotic discovered in the Soviet Union, possesses antitumor activity and is chemically related to adriamycin and daunorubicin.[1] The toxic effects of carminomycin observed in single-dose toxicologic studies in mice iv (1.5 - 4.5 mg/kg) and po (6.3 - 17.4 mg/kg), rats iv (1.0 - 3.5 mg/kg), dogs iv (0.05-1.0 mg/kg) and po (0.8 - 4.0 mg/kg), Rhesus monkeys po (2.0 - 12.0 mg/kg); and in multiple-dose studies in dogs iv (18 doses 0.03 - 0.25 mg/kg, 2X/week) and rats sc (9 doses 0.5 - 2.0 mg/kg, 3X/week), were dose-related and primarily manifested as suppression of hematopoesis. The changes observed consisted of reduction in numbers of bone marrow cells of the myeloid and erythroid series, and of lymphoid and/or reticuloendothelial cells. Other toxicities observed included nephrotoxicity, gastrointestinal toxicity, decreased spermatogenesis, decrease in size and activity of the prostate and seminal vesicles, and ovarian alterations. In general, these toxicities were reversible in surviving animals during 6-week to 4-month recovery periods. The LD_{50} values in mice iv and po were determined as 2.05 and 12.6 mg/kg respectively. Cardiotoxicity, similar to that seen with adriamycin (13 weekly doses of 2.0 mg/kg)[2] was not observed with carminomycin (13 weekly doses of 1.0 mg/kg).

1. Gause,G.F., Brazhnikova,M.G. and Shorin,V.A., Cancer Chemother. Rep. 58, 255 (1975).
2. Mettler,F.P., Young,D.M. and Ward,J.M., Cancer Res., 37, 2705 (1977).

474 CHANGE IN HEPATIC GLUTATHIONE (GSH) LEVELS IN MALE AND FEMALE F344 RATS TREATED WITH LOW PROTEIN AND DIETARY AFLATOXIN (AFB_1). K. D. Mainigi, Inst. of Comparative & Human Tox., Albany Med. Col., Albany, NY; and T. C. Campbell, Div. of Nutr. Sci., Cornell Univ., Ithaca, NY.

It has been proposed that the level of hepatic GSH may play an important role in determining the proportion of procarcinogenic aflatoxin dose that eventually binds to critical cellular macromolecules. The primary objectives of this study were to investigate the effects of different levels of protein intake and ingestion of hepatotoxic doses of AFB_1 on both, hepatic GSH levels and the induction of hepatic lesions. After acclimiation to a 20% casein (20C) agar-gel diet for 6 days, groups of weanling male (71g) and female (76g) rats were fed 5C or 20C diets with/without 2.5-5 ppm AFB_1 for 6 wks. Animals were sacrificed at selected intervals during this time period and liver GSH concentrations were determined. After the first 12 hrs, rats were always killed between 9 and 10 AM to minimize diurnal variations in GSH level. In terms of growth rate, females were quite resis-